Data Requirement:

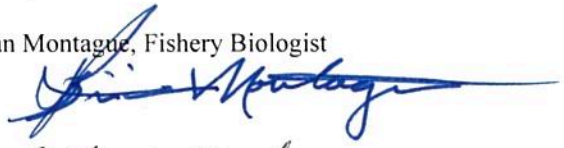
PMRA DATA CODE	
EPA DP Barcode	D305127
OECD Data Point	
EPA MRID	46462901
EPA Guideline	850.1300 (old FIFRA 72-4b)

Test material: Ethylene Thiourea **Purity:** 96.2%
Common name: ETU
Chemical name: IUPAC:Not reported
CAS name: Ethylenethiourea
CAS No.: Not reported
Synonyms: None reported

Primary Reviewer: Christie E. Padova **Signature:**
Staff Scientist, Dynamac Corporation **Date:** 4/8/05

QC Reviewer: Gregory Hess **Signature:**
Staff Scientist, Dynamac Corporation **Date:** 4/14/05

Primary Reviewer: Brian Montague, Fishery Biologist **Date:** February 2015
OPP/EFED/ERB - V



Secondary Reviewer(s): **Date:** February 2015
{EPA/OECD/PMRA}

Allen M. Vaughn 04/16/15

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 600016

Date Evaluation Completed: February 20, 2015

CITATION: Graves, W.C., M.A. Mank, and J.P. Swigert. 1995. Ethylene Thiourea (ETU): A Flow-Through Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*). Unpublished study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 129A-116. Study sponsored by Rohm & Haas Company, Spring House, PA. Sponsor Report No. 94RC-0001. Experimental start date October 21, 1994, and experimental termination date November 14, 1994. Final report issued January 3, 1995.

EXECUTIVE SUMMARY:

The 21-day-chronic toxicity of Ethylene Thiourea (ETU) to *Daphnia magna* was studied under flow-through conditions. Nominal concentrations were 0 (negative control), 0.25, 0.50, 1.0, 2.0, and 4.0 ppm a.i. (adjusted for purity). Mean-measured concentrations were <0.01 (<LOQ, control), 0.28, 0.54, 1.0, 2.0, and 4.1 ppm a.i., respectively, and were stable throughout the study.

After 21 days of exposure, cumulative adult mortality was 8% for the negative control group, compared to 10, 5, 8, 5, and 50% for the mean-measured 0.28, 0.54, 1.0, 2.0, and 4.1 ppm a.i. treatment groups, respectively. Although insufficient mortality occurred to calculate an LC_{50}, the 21-day LC_{50} for mortality was visually estimated to be approximately 4.1 ppm a.i., based on 50% mortality at this level. The LOEC for survival was determined to be 4.1 ppm a.i., and the NOEC was 2.0 ppm a.i.. All surviving daphnids appeared normal throughout the test. In addition, there were no male first-generation daphnids or ephippia produced during the study. Note, the mean-measured 4.1 ppm a.i. treatment group was excluded from all growth and reproduction statistical analyses due to the significant ($p<0.05$) reduction in adult survival.

By Day 21, mean surviving adult lengths were 3.8 and 3.8, 3.8, 3.7, 3.7, and 3.5 mm (study reported rounded treatment means) in the negative control and mean-measured 0.28, 0.54, 1.0, 2.0, and 4.1 ppm a.i. treatment groups, respectively. Terminal lengths were significantly reduced compared to the control at the 1.0 and 2.0 ppm a.i. treatment levels based on the reviewer's statistical verification of the reported raw data (William's test, $p<0.05$). Mean surviving adult dry weights were 0.52 and 0.50, 0.51, 0.48, 0.51, and 0.31 mg in the negative control and mean-measured 0.28, 0.54, 1.0, 2.0, and 4.1 ppm a.i. treatment groups, respectively. Terminal dry weights were not statistically reduced compared to the control at any treatment level included in the analysis (William's test, $p>0.05$). The NOEC for growth (based on the significant treatment-related reductions in terminal lengths) was 0.54 ppm a.i..

Eggs in the brood pouch were first observed in some adults on Day 6. In general, the percent of adults with eggs in their brood pouch during the reproduction period of the test (Days 6-21) in the negative control and 0.28 through 2.0 ppm a.i. treatment groups (81.1-100% on Days 10-21) appeared unaffected by the test material. However, the percent was decreased in the 4.1 ppm a.i. treatment group (45.7-80% on Days 10-17 and 19-21 and was 96% on Day 18) during the reproductive period. These observations suggest that the test material decreased the surviving adults' ability to produce successive broods in the highest treatment level tested. No statistically-significant differences in reproduction (mean number of live and dead or immobile offspring/adult/reproductive day) endpoints were observed between the control group and the 0.28, 0.54, 1.0, and 2.0 ppm a.i. treatment groups. Consequently, the NOEC for reproduction was 2.0 ppm a.i..

Note, EC_{50} values for growth and reproduction could not be calculated due to the statistically significant effect on adult survival at the 4.1 ppm a.i. treatment level and the lack of a 50% or greater effect at the lower treatment levels. Consequently, these values were visually estimated to be >2.0 ppm a.i. based on the mean-measured treatment concentrations.

This study is scientifically sound, and fulfills the guideline requirements for an aquatic invertebrate life cycle test with *Daphnia magna* (§ 72-4b). This study is classified ACCEPTABLE. Data obtained from this study are useful for risk assessment purposes.

Results Synopsis:

Test Organism Age (eg. 1st instar): First instar, <24 hours old
Test Type (Flow through, Static, Static Renewal): Flow-through

Mortality/Immobilization:

NOEC: 2.0 ppm a.i.
LOEC: 4.1 ppm a.i.
LC_{50}: Approx. 4.1 ppm a.i.

Length of Surviving Adults
NOEC: 0.54 ppm a.i.
LOEC: 1.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Dry Weight of Surviving Adults
NOEC: ≥2.0 ppm a.i.
LOEC: >2.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Mean No. Live Offspring/Adult/Repro. Day
NOEC: ≥2.0 ppm a.i.
LOEC: >2.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Mean No. Dead or Immob. Offspring/Adult/Repro. Day
NOEC: ≥2.0 ppm a.i.
LOEC: >2.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Endpoint(s) Affected: Mortality, reproduction, growth
Most sensitive endpoint(s): Growth (Length) of surviving adults

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study protocol was based on procedures outlined in the OECD Guidelines for Testing of Chemicals, No. 202 (1984) and ASTM Standard E 1193-87 (1991). Deviations from U.S. EPA FIFRA Guideline §72-4b or 850.1300 include:

The study design followed OECD Guidelines and does adhere to current EPA 850.1300 guidance. In this flow-through toxicity study, 10 daphnids per test compartment were maintained, with two replicate compartments per chamber, and two replicate chambers per concentration (total of 40 daphnids/concentration).

1. Concentrations did not bracket a 50% mortality level, but did produce 50 % mortality at the highest concentration.
2. Production of young per adult over 21 days (15 reproductive days) averaged 47.5 instead of recommended average of 60 per adult in the control daphnids. This may have been due to loss of 3 adults (8%) before day 17 (2 adults on day 16).
3. Pretest culture reproduction performance not provided, but no ephippia or signs of stress were reported.

This deviation does not affect the validity or acceptability of this study.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance

with the GLP Standards as published by OECD, ISBN 92-84-12367-9 (1982).

A. MATERIALS:

1. Test Material Ethylene Thiourea (ETU)

Description: White powder,

Lot No./Batch No.: HW02506EV

Purity: 96.2%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water was demonstrated by analytical determinations at 0, 7, 14, and 21 Days. Reviewer-calculated high-low ratios were 1.1-1.2, indicating stability during the course of the study.

Storage conditions of test chemicals: Ambient

OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound). The following OECD requirements were reported:

Water solubility: 20,000 mg/L at 30°C

Water stability: In sterile water - stable; in non-sterile water - stable <48 hours.

2. Test organism:

Species: *Daphnia magna*

Age of the parental stock: ≥14 days old

Source: In-house laboratory cultures

B. STUDY DESIGN:

1. Experimental Conditions

a. Range finding Study: None reported.

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: Period:	Continuous in-house cultures were maintained. Parental daphnids were isolated for a 14-day holding period.	The progeny from eleven adults were used in the test. Progeny were transferred with wide-bore pipettes to glass beakers and then moved from the beakers to the individual test chambers. Release was made below the water surface.
Conditions: (same as test or not)	Same as test, except that culture water was supplemented with selenium.	
Feeding:	*Daphnia* cultures were fed once daily with 0.15 mL YCT (mixture of yeast, Cerophyll, and trout chow) and 0.30 mL algae (*Selenastrum captricornutum*).	
Health: (any mortality observed)	No signs of disease or stress was observed during the holding period.	
Test condition: Static renewal/flow through:	Flow-through	The split flow accuracy (two replicate chambers) varied by no more than 10%.
Type of dilution system	Continuous flow diluter	Each test chamber received approx. 14 volume additions/24 hours.
Renewal rate for static renewal	N/A	The general operation of the diluter was checked visually at least twice daily during the test.
		For flow-through study: consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.
Aeration, if any	None reported	
		Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks should not be aerated.
Duration of the test	21 days	

Parameter	Details	Remarks *Criteria* *EPA requires 21 days for static renewal*
Test vessel Material: (glass/stainless steel) Size: growth/reproduction test: survival test: Fill volume: growth/reproduction test: survival test:	Test compartments were 500-mL glass beakers with Nytex screen attached to each end. Two compartments were suspended in Teflon-lined 8-L polyethylene test chambers filled with approx. 6.5 L of test water. The depth of the water was approx. 9.0 cm in each test compartment and 17 cm in each test chamber. The growth/reproduction and survival portions of the test were performed simultaneously in the same replicate test chambers/beakers (as per OECD guidance).	*1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics* *2. Size: 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable.* *OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 mL of medium in each vessel.*
Source of dilution water	Medium-hard freshwater was obtained from a well 45 m deep located on the laboratory premises. The well water was sand-filtered, aerated, filtered again, and UV-sterilized prior to use.	Results of periodic analysis of the dilution water (May 1994) for pesticides, organics, metals, and other inorganics were provided. *Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine Interval of water quality measurements	 140 mg/L as $CaCO_3$ 8.1-8.3 ≥7.9 mg/L (where 5.4 mg/L represents 60% saturation at 20°C) 19.9-20.3 °C <0.5 mg/L (well water analysis) Not specified (TDS: 248 ppm) Ca: 32800 ppb; Mg: 13.1 ppm; K: 6730 ppb; Na: 21200 ppb all others <LOD. <LOD (well water analysis) Not reported Temp. measured weekly in each chamber and continuously in one negative control replicate. pH - weekly in alternate replicates of each level. DO - daily during the first week and weekly thereafter in alternate replicates of each level. Hardness, alkalinity, and specific cond. - weekly in alternating replicates of the negative control.	The hardness and pH levels slightly exceeded the recommended EPA ranges, but were within OECD range requirements. ***hardness*** *160 to 180 mg/L as $CaCO_3$; OECD requires > 140 mg/L as $CaCO_3$* ***pH*** *7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours. OECD requires pH rang 6 - 9 and should not vary more than 1.5 units in any one test.* ***Dissolved Oxygen*** *Renewal: must not drop below 50% for more than 48 hours. Flow-through: ≥60% throughout test.* ***Temperature*** *20℃ ± 2℃. Must not deviate from 20℃ by more than 5℃ for more than 48 hours. OECD requires range 18 - 22°C; temperature should not vary more than ± 2°C OECD requires total organic carbon < 2 mg/L*
Number of organisms: growth/reproduction test: survival test:	40 daphnids/level 10 daphnids per test compartment, with two compartments per test chamber, and two test chambers per concentration level. (Not differentiated; same test chambers as above)	Did not follow US EPA recommended test design; OECD guidance was followed. *EPA requires 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.* *OECD requires minimum of 10 daphnids held individually for static tests. For flow-through tests, 40 animals divided into 4 groups of 10 animals at each test concentration.*

Parameter	Details	Remarks *Criteria*
Application rates nominal: measured:	0 (negative control), 0.25, 0.50, 1.0, 2.0, and 4.0 ppm a.i. (corrected for purity) <0.01 (<LOQ, control), 0.28, 0.54, 1.0, 2.0, and 4.1 ppm a.i.	Mean-measured recoveries were 100-112% of nominal. *EPA requires control(s) and at least 5 test concentrations; dilution factor not greater than 50%.* *OECD requires at least 5 test concentrations in a geometric series with a separation factor not exceeding 3.2.*
Solvent (type, percentage, if used)	N/A	 *EPA requires: solvent not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethyl formamide, triethylene glycol, methanol, acetone and ethanol. OECD requires ≤ 0.1 ml/L*
Lighting	16:8 hour light/dark cycle, with 30-minute transition periods.	Light intensity was approximately 464 lux at the surface of the water. *EPA/OECD requires: 16 hours light, 8 hours dark.*
Feeding	*Daphnia* were fed 3X daily with 0.3 mL YCT (mixture of yeast, Cerophyll, and trout chow) and 3.0 mL algae (*Selenastrum captricornutum*).	
Stability of chemical in the test system	Verified by analytical determinations at 0, 7, 14, and 21 Days. Reviewer-calculated high-low ratios were 1.1-1.2, indicating stability during the course of the study.	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	102 ± 4.6% Concurrent with sample analyses Not reported 0.01 ppm a.i.	Based on matrix samples fortified at 0.01, 0.20, 1.0, and 10.0 ppm a.i. and analyzed concurrently during sample analysis.
Positive control {if used, indicate the	N/A	

Parameter	Details	Remarks *Criteria*
chemical and concentrations}		
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Data end points measured (list)	- Survival and immobility of first-generation daphnids - Clinical signs of toxicity - Onset of reproduction, presence of eggs in the brood pouch, males or ephippia - Number of live and dead/immobile young produced - Number of live young/adult/reproductive day - Total length and dry weight of surviving first-generation daphnids	Only adult survival, growth (length and dry weight), number of live young/adult/reproductive day, and number of dead and/or immobile young/adult/reproductive day were statistically assessed for treatment related differences compared to the control.
		EPA requires: *- Survival of first-generation daphnids,* *- Number of young produced per female,* *- Dry weight (recommended) and length (required)* of each first generation daphnid alive at the end of the test,* *- Observations of other effects or clinical signs.* **current requirement until the Agency provides specific guidance indicating otherwise (Pesticide Rejection Rate Analysis, p. 132).*
Observation intervals	Daphnids were observed daily. With the onset of reproduction, offspring production was recorded then discarded every Monday, Wednesday, and Friday and at test termination. Body length and dry weights were determined at test termination.	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY/IMMOBILITY: (Based on the study reported results)

After 21 days of exposure, the cumulative mortality was 8% for the negative control group, compared to 10, 5, 8, 5, and 50% for the 0.28, 0.54, 1.0, 2.0, and 4.1 ppm a.i. test groups, respectively. Although insufficient mortality occurred to calculate an LC_{50}, the 21-day LC_{50} for mortality was estimated to be approximately 4.1 ppm a.i., based on 50% mortality at this level. Using 2 X 2 contingency tables, the LOEC for survival was determined to be 4.1 ppm a.i., and the NOEC was 2.0 ppm a.i..

All surviving daphnids (all control and test levels) appeared normal throughout the test. There were no male first-generation daphnids or ephippia produced during the study.

Table 1: Effect of Ethylene Thiourea (ETU) on Growth and Survival of Daphnia sp.

Treatment, ppm a.i. Mean-measured (and Nominal) Concn.	Mortality (Dead)		Mean Dead or Immob. Young/Adult/-Repro. Day	Mean Live Young/Adult/-Repro. Day	Length, mm	Dry Weight, mg
	No. Dead	%				
Negative control	3	8	0.026	3.3	3.8	0.52
0.28 (0.25)	4	10	0.017	3.4	3.8	0.50
0.54 (0.50)	2	5	0.016	3.3	3.8	0.51
1.0 (1.0)	3	8	0.033	3.1	3.7	0.48
2.0 (2.0)	2	5	0.037	3.1	3.7	0.51
4.1 (4.0)	20*	50	0.053[1]	1.7[1]	3.5[1]	0.31[1]
NOEC, ppm a.i.	2.0		2.0	2.0	2.0	2.0
LOEC, ppm a.i.	4.1		4.1	4.1	4.1	4.1
MATC, ppm a.i.	2.9		ND	ND	ND	ND
LC_{50}/EC_{50}, ppm a.i.	Approx. 4.1		ND	ND	ND	ND

* Statistically different from control group.
[1] Data not included in statistical analysis due to effect on survival at this level; however, data were visibly different from control group.
ND - Not determined.

B. EFFECT ON REPRODUCTION AND GROWTH: (Based on the study reported results)

Eggs in the brood pouch were reported to be first observed in some adults on Day 6. In general, most adults contained eggs in their brood pouch during the reproduction period of the test (Days 6-21). No clear dose-response was observed in the numbers of adults holding eggs in their brood pouch.

No statistically-significant differences in reproductive (mean number of live and dead or immobile offspring/adult/reproductive day) or growth (length and dry weight) endpoints were observed between the control group and the 0.28, 0.54, 1.0, and 2.0 ppm a.i. treatment groups.

The 4.1 ppm a.i. group was excluded from statistical analyses due to a significant reduction in survival; however, based on visual interpretation of the data, a treatment-related difference from the control group was observed at all reproductive and growth endpoints at the 4.1 ppm a.i. level.

C. REPORTED STATISTICS:

Statistical Method: Insufficient immobilization and/or mortality occurred in the highest test concentration to calculate an LC/EC_{50}. However, since 50% mortality occurred at the 4.1 ppm a.i., the LC_{50} was estimated to be 4.1 ppm a.i.. Survival data were also evaluated using 2 X 2 contingency tables to identify treatment groups statistically different from the negative control. Reproductive and growth data were evaluated for homogeneity of variances using the Bartlett's test and for normality using a goodness of fit test (e.g., Chi-square test). ANOVA was then used to determined statistically-significant differences among the experimental groups, and those treatments statistically different from the control group were identified using the Bonferroni t-test. It was noted that the mean-measured 4.1 ppm a.i. treatment group growth and reproduction data were excluded from the statistical analyses due to the significant effect on adult survival.

All statistical tests were performed using SPSS/PC Version 2.0 or TOXSTAT Version 3.2 statistical software and mean-measured concentrations.

Mortality/immobilization:
NOEC: 2.0 ppm a.i.
LOEC: 4.1 ppm a.i.
LC_{50}: Approx. 4.1 ppm a.i.

Length of Surviving Adults
NOEC: 2.0 ppm a.i.
LOEC: 4.1 ppm a.i.

Dry Weight of Surviving Adults
NOEC: 2.0 ppm a.i.
LOEC: 4.1 ppm a.i.

Mean No. Live Offspring/Adult/Repro. Day
NOEC: 2.0 ppm a.i.
LOEC: 4.1 ppm a.i.

Mean No. Dead or Immob. Offspring/Adult/Repro. Day
NOEC: 2.0 ppm a.i.

LOEC: 4.1 ppm a.i.

Endpoint(s) Affected: Mortality, reproduction, growth
Most sensitive endpoint(s): Mortality, reproduction, growth

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer attempted to determine an LC_{50} value using the binomial, moving average, and probit methods. These methods were unsuccessful due to the lack of a linear dose-response. Consequently, the 21-day LC_{50} was visually estimated based on th reported mortality at the highest treatment level tested (50% at 4.1 ppm a.i. treatment level). After confirming normality and homogeneity of variances, the NOEC values for survival and growth (length and dry weight) of adults compared to the negative control were determined using ANOVA and William's multiple comparison test. The mean-measured 4.1 ppm a.i. treatment group (the highest concentration tested) was excluded from the growth and reproduction statistical analyses due to a statistically significant reduction in adult survival by 21 days. The NOEC for reproduction (mean number of live young per adult per reproductive day) data were determined to be normally distributed and the variances were homogeneous. These data were assessed for treatment-related reductions compared to the control using ANOVA and William's multiple comparison test. The NOEC based on the number of dead or immobile young per adult per reproductive day data also met the assumptions of ANOVA and were assessed for treatment-related increases compared to the control group using ANOVA and Dunnett's test. The above statistical analyses were performed via TOXSTAT statistical software. EC_{50} values for growth and reproduction could not be calculated due to the statistically significant effect on adult survival at the 4.1 ppm a.i. treatment level and the lack of a 50% or greater effect at the lower treatment levels. Consequently, these values were visually estimated to be >2.0 ppm a.i. based on the mean-measured treatment concentrations. The NOEC for dry weight and reproduction were determined to be greater than or equal to the 2.0 ppm a.i. level because effects could not be evaluated at the 4.1 ppm a.i. level due to excessive mortality and exclusion of this level from these statistical analyses. The analysis for adult length using Williams test and excluding the 4.1 treatment group, due to mortality issues, shows significant effects at treatment levels of 1.0 and 2.0 ppm treatment levels with no effect observed at 0.54 ppm measured concentration level. All the above analyses were performed in terms of the mean-measured treatment concentrations.

Mortality/Immobilization:
NOEC: 2.0 ppm a.i.
LOEC: 4.1 ppm a.i.
LC_{50}: Approx. 4.1 ppm a.i.

Length of Surviving Adults
NOEC: 0.54 ppm a.i.
LOEC: 1.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Dry Weight of Surviving Adults
NOEC: ≥2.0 ppm a.i.
LOEC: >2.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Mean No. Live Offspring/Adult/Repro. Day
NOEC: ≥2.0 ppm a.i.
LOEC: >2.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Mean No. Dead or Immob. Offspring/Adult/Repro. Day
NOEC: ≥2.0 ppm a.i.
LOEC: >2.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Endpoint(s) Affected: Mortality, reproduction, growth
Most sensitive endpoint(s): Growth (Length) of surviving adults

E. STUDY DEFICIENCIES:

The study design followed OECD Guidelines and EPA 850.1300 guidance. In this flow-through toxicity study, 10 daphnids per test compartment were maintained, with two replicate compartments per chamber, and two replicate chambers per concentration (total of 40 daphnids/concentration). This number of daphnids differs slightly from earlier 72-4 guidance, but the study was conducted as the new 850 guidelines for flow through testing were being finalized and the 72-4 guideline referred to static renewal testing only.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions for adult survival were identical to those of the study authors (NOEC, LOEC and LC50 were 2.0, 4.1, and 4.1 ppm a.i., respectively). The reviewer-determined NOEC and LOEC for terminal adult length were 0.54 and 1.0 ppm a.i., respectively and were two treatment levels lower than those of the study authors, 2.0 and 4.1 ppm a.i., respectively. The observed differences were attributed to the different statistical methods used. The study authors reported the LOEC for growth (length and dry weight) and reproduction (both number of live and dead or immobile young per adult per reproductive day) to be 4.1 ppm a.i. based on visual assessment of the data collected from the surviving daphnids from the 4.1 ppm a.i. treatment group, however, data from this treatment group were excluded from the reported statistical analyses. The reviewer-determined LOEC values for adult dry weight and reproduction were estimated to be greater than the second highest treatment level tested, e.g., >2.0 pm a.i., since the highest treatment level tested (4.1. ppm a.i.) was excluded from the statistical analyses due to a significant ($p < 0.05$) reduction in adult survival by 21 days. For those endpoints discussed above, the more conservative reviewer-determined toxicity values are reported in the EXECUTIVE SUMMARY and CONCLUSION section of this DER.

G. CONCLUSIONS:

The study is scientifically sound, and fulfills the guideline requirements for an aquatic invertebrate life cycle test with the *Daphnia magna* (§72-4b) using Ethylene Thiourea (ETU). This study is classified as ACCEPTABLE. Mortality was affected by treatment at the mean-measured 4.1 ppm a.i. level while growth (adult length) was affected at the 1.0 and 2.0 ppm a.i. treatment levels. Consequently, the NOEC for adult survival and growth were 2.0 and 0.54 ppm a.i., respectively. Reproduction was not significantly affected at treatment levels ≤2.0 ppm a.i., by 21 days, thus the NOEC for reproduction was ≥2.0 ppm a.i..

Mortality/Immobilization:
NOEC: 2.0 ppm a.i.
LOEC: 4.1 ppm a.i.
LC_{50}: Approx. 4.1 ppm a.i.

Length of Surviving Adults
NOEC: 0.54 ppm a.i.

LOEC: 1.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Dry Weight of Surviving Adults
NOEC: ≥2.0 ppm a.i.
LOEC: >2.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Mean No. Live Offspring/Adult/Repro. Day
NOEC: ≥2.0 ppm a.i.
LOEC: >2.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Mean No. Dead or Immob. Offspring/Adult/Repro. Day
NOEC: ≥2.0 ppm a.i.
LOEC: >2.0 ppm a.i.
EC_{50}: >2.0 ppm a.i.

Endpoint(s) Affected: Mortality, reproduction, growth
Most sensitive endpoint(s): Growth (Length) of surviving adults

III. REFERENCES:

Organization for Economic Cooperation and Development. 1984. Guideline 202: *Daphnia* sp., *Acute Immobilization and Reproduction Test*.

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APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

TOXANAL RESULTS (LC50): Note: 50% mortality at highest Dose of 4.1 PPM not a good fit for any of the 3 tests below

THE NUMBER OF ORGANISMS USED IS TOO LARGE TO ALLOW CALCULATION
OF THE BINOMIAL PROBABILITY. THE LC50 CALCULATIONS ARE UNAFFECTED.

NOTE TO REVIEWER: THIS DATA SET DOES NOT MEET
THE CRITERIA ESTABLISHED BY THE COMMITTEE ON
METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS
BECAUSE NO PERCENT DEAD IS GREATER THAN 65 PERCENT.

NEITHER THE BINOMIAL TEST NOR THE MOVING AVERAGE METHOD
CAN GIVE ANY RESULTS FOR THIS DATA. EITHER THE HIGHEST CONCENTRATION
KILLED LESS THAN 50 PERCENT OR THE LOWEST KILLED MORE THAN 50.
IF THE PROBIT SLOPE IS NEGATIVE, ENTER DATA AGAIN USING NUMBER
ALIVE INSTEAD OF NUMBER DEAD.

BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET
45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	2.397797	4.611539	3.139019E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.853962
95 PERCENT CONFIDENCE LIMITS =-1.016865 AND 4.724788
LC50 = 6.886
95 PERCENT CONFIDENCE LIMITS = 2.182016 AND +INFINITY
LC10 = 1.422157
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

TOXSTAT RESULTS (NOEC& LOEC): Growth, Survival and Reproduction

Survival by day 21 (ppm a.i.)

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	62.333	12.467	10.441
Within (Error)	18	21.500	1.194	
Total	23	83.833		

Critical F value = 2.77 (0.05,5,18)

Since F > Critical F REJECT Ho:All groups equal

Survival by day 21 (ppm a.i.)

File: 2901md Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	9.250	9.250		
2	0.28	9.000	9.000	0.324	
3	0.54	9.500	9.500	-0.324	
4	1.0	9.250	9.250	0.000	
5	2.0	9.500	9.500	-0.324	
6	4.1	5.000	5.000	5.500	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

Survival by day 21 (ppm a.i.)
File: 2901md Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.28	4	1.862	20.1	0.250
3	0.54	4	1.862	20.1	-0.250
4	1.0	4	1.862	20.1	0.000
5	2.0	4	1.862	20.1	-0.250
6	4.1	4	1.862	20.1	4.250

Survival by day 21 (ppm a.i.)
File: 2901md Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	9.250	9.250	9.300
2	0.28	4	9.000	9.000	9.300
3	0.54	4	9.500	9.500	9.300
4	1.0	4	9.250	9.250	9.300
5	**2.0**	**4**	**9.500**	**9.500**	**9.300**
6	4.1	4	5.000	5.000	5.000

Survival by day 21 (ppm a.i.)

File: 2901md Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	9.300				
0.28	9.300	0.065		1.73	k= 1, ν=18
0.54	9.300	0.065		1.82	k= 2, ν=18
1.0	9.300	0.065		1.85	k= 3, ν=18
2.0	**9.300**	**0.065**		**1.86**	**k= 4, ν=18**
4.1	5.000	5.499	*	1.87	k= 5, ν=18

s = 1.093

Note: df used for table values are approximate when ν > 20.

Lengths of Surviving adults (mm; Day 21; ppm a.i.)

File: 2901ld Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.101	0.025	3.125
Within (Error)	181	1.497	0.008	
Total	185	1.598		

Critical F value = 2.45 (0.05,4,120)

Since F > Critical F REJECT Ho:All groups equal

Lengths of Surviving adults (mm; Day 21; ppm a.i.)
File: 2901ld Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	3.757	3.757		
2	0.28	3.779	3.779	-1.070	
3	0.54	3.751	3.751	0.263	
4	1.0	3.714	3.714	2.080	
5	2.0	3.725	3.725	1.537	

Bonferroni T table value = 2.27 (1 Tailed Value, P=0.05, df=120,4)

Lengths of Surviving adults (mm; Day 21; ppm a.i.)
File: 2901ld Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	37			
2	0.28	36	0.048	1.3	-0.022
3	0.54	38	0.047	1.2	0.005
4	1.0	37	0.047	1.3	0.043
5	2.0	38	0.047	1.2	0.032

Lengths of Surviving adults (mm; Day 21; ppm a.i.)
File: 2901ld Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	37	3.757	3.757	3.768
2	0.28	36	3.779	3.779	3.768
3	**0.54**	**38**	**3.751**	**3.751**	**3.751**
4	1.00	37	3.714	3.714	3.719

5	2.0	38	3.725	3.725	3.719

Lengths of Surviving adults (mm; Day 21; ppm a.i.)
File: 2901ld Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	3.768				
0.28	3.768	0.519		1.66	k= 1, ν=181
0.54	**3.751**	**0.259**		**1.73**	**k= 2, ν=181**
1.0	3.719	1.770	*	1.75	k= 3, ν=181
2.0	3.719	1.782	*	1.77	k= 4, ν=181

s = 0.091
Note: df used for table values are approximate when ν > 20.

Dry weight of surviving adults (mg; Day 21; ppm a.i.)
File: 2901wd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.028	0.007	1.400
Within (Error)	181	0.909	0.005	
Total	185	0.938		

Critical F value = 2.45 (0.05,4,120)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Dry weight of surviving adults (mg; Day 21; ppm a.i.)
File: 2901wd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.517	0.517		
2	0.28	0.504	0.504	0.777	
3	0.54	0.508	0.508	0.543	
4	1.0	0.480	0.480	2.236	
5	2.0	0.508	0.508	0.543	

Bonferroni T table value = 2.27 (1 Tailed Value, P=0.05, df=120,4)

Dry weight of surviving adults (mg; Day 21; ppm a.i.)
File: 2901wd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	37			
2	0.28	36	0.038	7.3	0.013
3	0.54	38	0.037	7.2	0.009
4	1.0	37	0.037	7.2	0.037
5	2.0	38	0.037	7.2	0.009

Dry weight of surviving adults (mg; Day 21; ppm a.i.)
File: 2901wd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	ORIGINAL	TRANSFORMED	ISOTONIZED

	IDENTIFICATION	N	MEAN	MEAN	MEAN
1	neg control	37	0.517	0.517	0.517
2	0.28	36	0.504	0.504	0.506
3	0.54	38	0.508	0.508	0.506
4	1.0	37	0.480	0.480	0.494
5	**2.0**	**38**	**0.508**	**0.508**	**0.494**

Dry weight of surviving adults (mg; Day 21; ppm a.i.)
File: 2901wd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	0.517				
0.28	0.506	0.652		1.66	k= 1, ν=181
0.54	0.506	0.661		1.73	k= 2, ν=181
1.0	0.494	1.374		1.75	k= 3, ν=181
2.0	**0.494**	**1.383**		**1.77**	**k= 4, ν=181**

s = 0.071
Note: df used for table values are approximate when ν > 20.

No. Dead/Immobile young/adult/repro. day by 21 days
File: 2901did Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.129	0.032	0.800
Within (Error)	15	0.599	0.040	
Total	19	0.728		

Critical F value = 3.06 (0.05,4,15)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

No. Dead/Immobile young/adult/repro. day by 21 days

File: 2901did Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.258	0.258		
2	0.28	0.168	0.168	0.636	
3	0.54	0.163	0.163	0.672	
4	1.0	0.322	0.322	-0.460	
5	**2.0**	**0.363**	**0.363**	**-0.742**	

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

No. Dead/Immobile young/adult/repro. day by 21 days

File: 2901did Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.28	4	0.334	129.6	0.090
3	0.54	4	0.334	129.6	0.095
4	1.0	4	0.334	129.6	-0.065
5	**2.0**	**4**	**0.334**	**129.6**	**-0.105**

No. Live young/adult/repro. day by 21 days (ppm a.i.)

File: 2901rd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.268	0.067	0.404
Within (Error)	15	2.497	0.166	
Total	19	2.766		

Critical F value = 3.06 (0.05,4,15)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

No. Live young/adult/repro. day by 21 days (ppm a.i.)

File: 2901rd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	3.275	3.275		
2	0.28	3.375	3.375	-0.347	
3	0.54	3.325	3.325	-0.174	
4	1.0	3.125	3.125	0.521	
5	2.0	3.075	3.075	0.694	

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

No. Live young/adult/repro. day by 21 days (ppm a.i.)

File: 2901rd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	REPS	NUM OF (IN ORIG. UNITS)	Minimum Sig Diff	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4				
2	0.28	4		0.680	20.8	-0.100
3	0.54	4		0.680	20.8	-0.050
4	1.0	4		0.680	20.8	0.150
5	2.0	4		0.680	20.8	0.200

No. Live young/adult/repro. day by 21 days (ppm a.i.)

File: 2901rd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	3.275	3.275	3.325
2	0.28	4	3.375	3.375	3.325
3	0.54	4	3.325	3.325	3.325
4	1.0	4	3.125	3.125	3.125
5	**2.0**	**4**	**3.075**	**3.075**	**3.075**

No. Live young/adult/repro. day by 21 days (ppm a.i.)

File: 2901rd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	3.325				
0.28	3.325	0.173		1.75	k= 1, ν=15
0.54	3.325	0.173		1.84	k= 2, ν=15
1.0	3.125	0.520		1.87	k= 3, ν=15
2.0	**3.075**	**0.693**		**1.88**	**k= 4, ν=15**

s = 0.408

Note: df used for table values are approximate when ν > 20.